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Laboratory refugia preferences of the brown widow spider, *Latrodectus geometricus* (Araneae: Theridiidae)

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Abstract. During collecting episodes in urban southern California, brown widow spiders, *Latrodectus geometricus* C. L. Koch, 1841, were observed to reside most often in specific locations such as acute angles within patio furniture or on the underside of railings. In this laboratory study, we tested several variables to determine which factors brown widow females may use for web site selection. All tests were conducted with corrugated cardboard refugia in enclosed dome-shaped insect cages. Brown widows were found to significantly prefer: 1) 30° angles compared to 60° or 90° angles, 2) cavities of 75 and 100 mm depth compared to 25 or 50 mm depth, 3) cavities lined with single-faced corrugated cardboard compared to smooth surfaces, and 4) refuges that had previously housed a brown widow compared to similar but previously unoccupied refugia. These data provide unusually detailed information about microhabitat selection by an urban pest, with possible implications for the pest control industry for eradication or for home owners to avoid envenomations.

Keywords: Synanthropy, urban entomology, urban pest

The brown widow spider, *Latrodectus geometricus* C. L. Koch, 1841, is non-native to North Ameriea (Garb et al. 2004). Its first North American establishment was in Florida in 1935 (Pearson 1936) where it still exists. In the early 21st century, *L. geometricus* greatly expanded its range, colonizing the southeastern Gulf Coast states (Brown et al. 2008) and southern California (Vineent et al. 2008). In southern California and possibly elsewhere, the brown widow spider has become a major nuisance pest such that it is now one of the most common spiders found around homes and parks in urban areas.

Field collections by Vetter et al. (2012a,b) enabled the authors to reliably identify locations where brown widows would be found. Spiders appeared to prefer refuges on the undersides of solid horizontal cover with acute angles being favored over obtuse angles (Fig. 1). This predilection for tight spaces has allowed the brown widow to preferentially infest the undersides of inexpensive plastic patio furniture, which has many crisscrossing, vertical supports to strengthen seats or tables, offering a multitude of refuge sites. The undersides of curved rims on the margins of plant pots and recessed handles on plastic trash cans are also preferred brown widow habit. Additionally, brown widows were plentiful under wooden picnic tables with the legs producing a right angle attachment to the tabletop but not so common under smooth concrete tables with gently curved leg supports. Situations like these put brown widow females in close association with humans, possibly increasing the potential of accidental envenomation.

Studies of refugia preferences of pests such as cockroaches (Koehler et al. 1994) have aided in developing control measures to construct traps to manage populations. In the laboratory, Vetter & Rust (2008) showed that recluse spiders (*Loxosceles reclusa* Gertsch & Mulaik, 1940, *L. laeta* (Nicolet, 1849)) preferred vertically oriented refugia rather than horizontal ones and preferred refugia containing silk of previous congenerics over refugia without silk, but had no definitive preference for cavity size among the sizes that were tested. Stropa (2010) offered various refugia placed in leaf litter where *Loxosceles gaucho* Gertsch, 1967 showed preference for refugia with acute angular internal walls rather than walls that were obtuse, right-angled or smoothly curved. Carrel (2015) demonstrated in the laboratory that spiderlings of *Kukulcania hibernalis* (Hentz, 1842) shifted to different sized holes drilled in wooden dowels as they grew over a 24-week period. Here we examine the preferences of female brown widow spiders for refugia characteristics in laboratory assays. Although this research may lend itself to practical use for pest control, it was performed primarily to determine whether brown widows assess structures for refuge suitability and show preference for some locations over others, and if so, whether general selection criteria could be identified.

METHODS

Spiders.—Brown widow spiders were collected from urban areas in spring and early summer in 2014 and 2015 (Orange County) and the city of Riverside (Riverside County), both in southern California, USA. Spiders were maintained individually in 163-ml plastic food cups (soufflé cups, First Street, Amerifoods Trading Co., Los Angeles, California) with plastic lids. A Y-shaped piece of fiberboard (2 mm wide with two legs of 35 mm length and one leg of 52 mm length) was placed inside the cup to act as a substrate for web attachment. Spiders were offered a mealworm (*Tenebrio molitor*) every 5 to 14 days, an amount that was sufficient to prevent spider death from starvation. We only tested subadult or mature females for refuge preferences.

General assay specifications.—Spiders were individually tested in insect cages (Fig. 2; Bug Dorm, BD2120F MegaView Science, Taiwan). The spiders were tested in the University of California Riverside Insectary and Quarantine Facility in a room with a south-facing window covered with unwaxed brown paper. The window did not receive direct sunlight. Average room temperature was 24.9 ± 0.4 °C and relative humidity was 39.0 ± 7.7 %. Lights were turned on when setting up an experiment, releasing spiders, checking spider position or

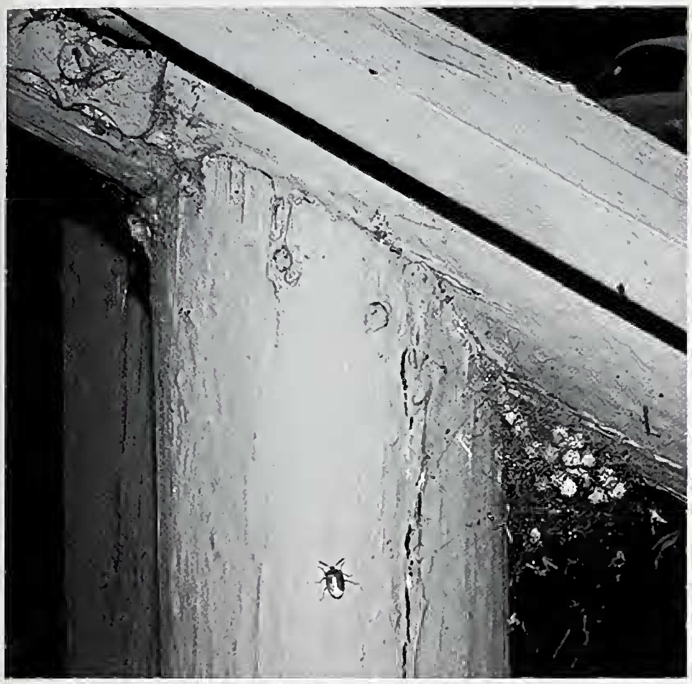

Figure 1.—A railing at a condominium complex showing the preference of the brown widow for acute vs. obtuse angles. In this picture, at least 17 egg sacs were collected from within the acute angle.

cleaning out cages for the next set of trials. A small piece of cardboard was placed on the bottom of the insect cage (to protect the thin plastic bottom) and a wooden dowel was placed on the cardboard such that it held cardboard refugia being tested at the top of the dome by wedging it in place (Fig. 2). This

Figure 2.—The insect cage (60 × 60 × 65 cm) with a wooden dowel positioned on a piece of cardboard at the bottom which forcibly wedges the test cardboard at the top, flush against the mesh such that the brown widow could not choose the space at the top of the insect cage. To initiate the assay, a cup containing the spider was uncapped and placed at the base of the dowel, near twilight.

tight-fitting design also prevented spiders from seeking refuge in the small space above the test cardboard shape. Therefore, when deployed, the open portion of the cardboard refugia faced downward and the spider could elect to crawl up the dowel and use one of three sites for refugia: the cardboard cavity, the vertical folds of the insect cage or the interface of the upper cardboard and mesh. Experimental cardboard refugia were constructed with white glue (Elmer's School Glue, Elmer's Products, Inc., Westerville, Ohio). In addition, when pieces of cardboard refugia required stapling, the cardboard was stapled from the inside of the strip so the piercing ends of metal were outside the cavity and then flattened with pliers in case the staples influenced site selection; this preference was examined in the first assay.

In all tests except the second pre-occupancy assay (see below), spiders were released around twilight by uncapping and placing the open cup near the base of the dowel without forcing the spider out of the cup. This allowed spiders to leave the cup on their own accord during their nocturnal activity period. In most assays, eight spiders were tested at one time, if there were sufficient numbers. To minimize disturbance, the location of the spider in the cardboard was detected using a flashlight and a telescoping mirror. Both the spiders and the cardboard refugia were used only once in a particular assay even if spiders did not take refuge within the test structure. When an individual assay was terminated, the wooden dowels and inside walls of the insect cages were wiped with a dry paper towel to remove noticeable silk.

The assays are presented here sequentially. However, in order to maximize the use of spiders before senescence reduced the pool of specimens, often the different categories of assays were run consecutively, building up sample size contemporaneously, as opposed to completing one assay before starting the next one. Therefore, information determined in one assay, which might appear useful in the design of subsequent assays, often was not available for us to incorporate at the time of initial assay undertaking.

Angle preference assay.—A 25-mm × 500-mm strip of corrugated cardboard was configured into a geometric shape with leg lengths of approximately 9, 15.3 and 18 cm, and a small length of overlap for stapling, creating a triangle with 30°, 60° and 90° angles (Fig. 3). The stapled overlapping end was distributed evenly among angles in case staple texture influenced refugia choice. The triangle was glued to a 22.5-cm square piece of corrugated cardboard. One angle was placed pointing toward one edge and the other two angles toward the side edges in equal numbers such that north and south angles were equally represented in the samples tested for each of the three angle categories. The cardboard square with the triangle facing downward was wedged against the roof of the insect cage with a wooden dowel (19-mm diameter × 56-cm length) (Fig. 2).

Cages were checked daily for three consecutive days to determine the location of the spider, although because of site fidelity the first day's position was sufficient for analysis. Data were collected on whether a spider chose an angle in the cardboard triangle, which angle was chosen, and whether the chosen angle had staples. In this assay, 48 spiders were tested.

Depth preference and site fidelity assay.—In this assay, spiders were offered a cardboard box with four compartments of

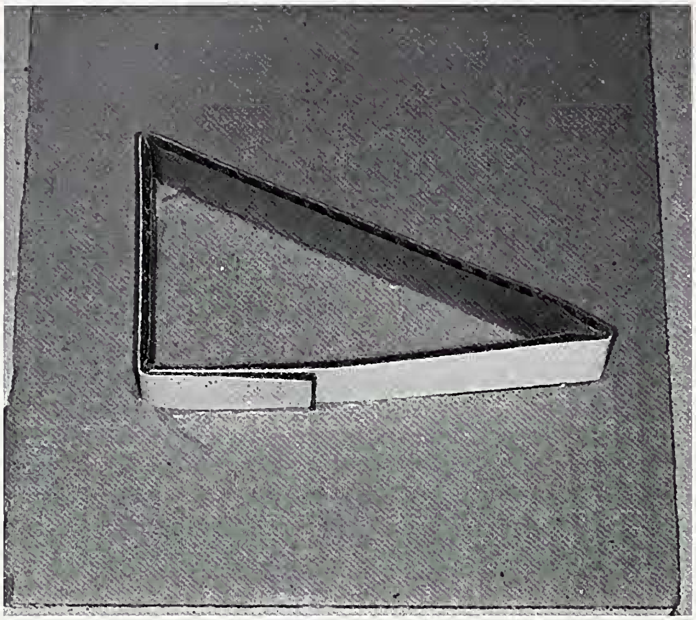

Figure 3.—The triangle glued to a cardboard square. The square was inverted when put in place with the opening of the triangle facing downward.

depths of 25, 50, 75 and 100 mm (Fig. 4). Cardboard shipping boxes for fluorescent light bulbs were obtained from a local lighting eompany. The internal cross-sectional dimensions of the boxes were approximately 140 × 185 mm. Boxes were eut into 100 mm sections with a table saw, a piece of cardboard was glued to the bottom and a cardboard cross was inserted in the box to form four subequal cavities of approximately 60-mm width × 90-mm length. U-shaped cardboard inserts were then individually cut and slid into cavities to produce depths of 25, 50, and 75 mm (Fig. 4) and were held in place by snugness of fit without glue. The 100-mm depth cavity required no insert. The order of the depths was randomized starting in the lower left hand corner, moving clockwise in order

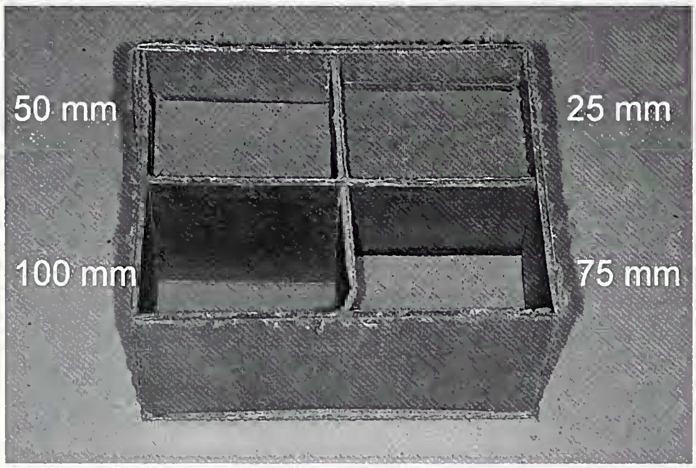

Figure 4.—A 100 mm deep box, separated into four sections with cardboard inserts placed in three of the sections of depths of 25, 50 and 75 mm. Only the flat bottom of the inverted U-shaped insert is visible. The box was inverted when put in place with the opening of the box facing downward.

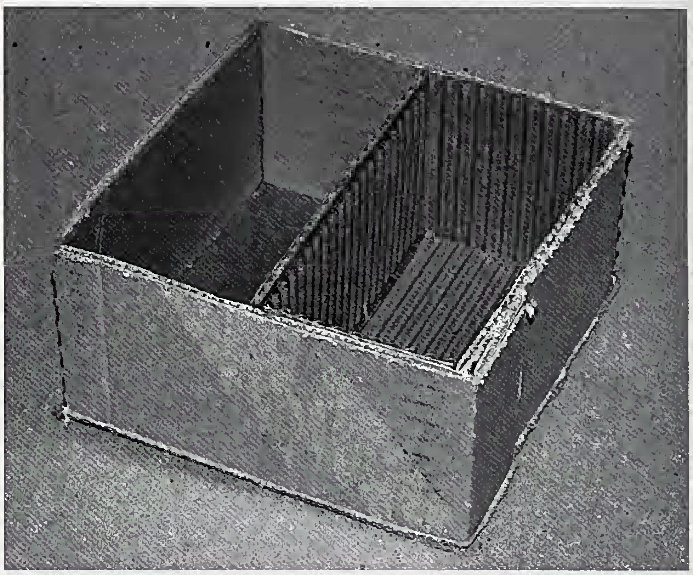

Figure 5.—A 75 mm deep box, bisected with one cavity lined with single-faced corrugated cardboard. The box was inverted when put in place with the opening of the box facing downward.

that one depth would have randomly chosen depth cavities as adjacent neighbors from box to box. To effect the test, the box was secured against the roof of the insect cage with a wooden dowel (19-mm diameter × 46-cm length) whieh was placed at the middle of the box resting on the cross-section of the cross insert so that the spider had equal chanee of accessing any of the four cavities. In this assay, 36 spiders were tested.

To test site fidelity more thoroughly, in this depth assay spiders were checked every day for a week to determine if any changes in daily position occurred, either from one cavity to another or from the initial chosen corner to another within a cavity.

Surface roughness assay.—We tested whether brown widows preferred a rough or smooth eardboard surface on the inside of their cavity. Fluorescent lighting boxes with a 150-mm square eross section were cut into 75-mm sections and a cardboard bottom was glued to each. A cardboard divider created two

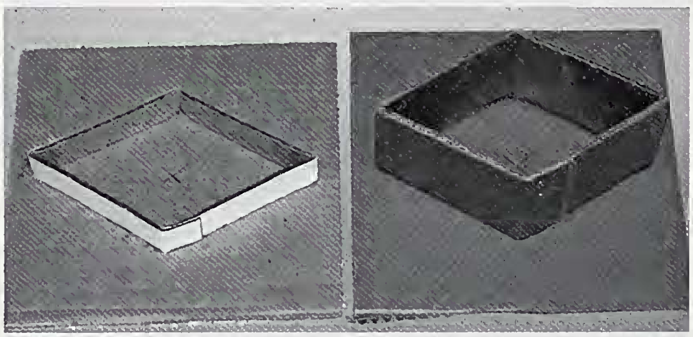

Figure 6.—The parallelogram of 60° and 120° angles and 25-mm height in the first previous-occupancy assay (left), which was placed at the top of the insect cage. The squares were inverted when put in place with the opening of the parallelogram facing downward. In the second assay, the walls of the parallelogram were increased to 75 mm (right) and the parallelogram was placed directly over the cup with the spider, ensuring that the spider would only make a choice within the parallelogram interior.

subequal cavities of 70-mm width × 140-mm length. Pieces of single-faced B-flute corrugated eardboard (2-mm high corrugations at 11 corrugations per 7 cm of length) were used to line the bottom and walls of one of the cavities with the corrugations facing outward, creating a rugose surface (Fig. 5). The other cavity was left unaltered with smooth walls and bottom. The completed paired cavities were wedged into the roof of the insect cage with a wooden dowel (19-mm diameter × 49-cm length). The eavity with corrugations was equally oriented at the four cardinal compass points successively between runs. Spiders were checked every day for three days. In this assay, 48 spiders were tested.

Pre-occupancy assay #1.—Spiders were tested to see if prior occupancy by another brown widow would influence choice. A 25-mm wide strip of cardboard was formed into a parallelogram with two 60° and two 120° angles. Staples were placed in one of the 120° angles such that the two 60° angles would be identical (Fig. 6, left). The length of each leg was 11.5 cm. Results from earlier tests indicated that the spiders would preferentially choose one of the 60° angles. The parallelogram was arranged such that the 60° angles alternated between north-south or east-west orientation in successive runs on top of the same dowel used in the angle preference assay. In order for a cardboard refugium to be used by the second cohort of spiders, the first occupant needed to be found in the same location for each of the 7 days such that only one location should show signs of occupancy. Refugia were checked for 7 consecutive days with no spider in a refugium switching position from the first day. On the morning of the seventh day, spiders were gently forced out with a probe, while attempting to minimally disrupt the web structure. The cardboard refuge was repositioned in the same orientation as in the first portion of the assay. A second set of spiders was then released into evacuated test arenas at dusk. The position of the second cohort of spiders was recorded for 7 consecutive days. In this portion of the assay, 32 spiders were tested in the first cohort with 15 being tested in the second cohort.

Preoccupancy assay #2.—The premise of the above experiment was repeated, however, to increase the percentage of spiders choosing a location in the cardboard refugium in this assay in eomparison to the previous assay, the width of the strip of cardboard was increased from 25 to 75 mm (Fig. 6, right) and instead of having the cardboard refugia at the top of a dowel, the cup with the spider was opened and the cardboard refugia was placed directly over the cup located on the floor of the insect cage. This allowed the spider to leave the 60-mm tall cup and choose an angle inside the 75-mm high refugium. The spiders were checked each day for the following week, forced out on the seventh morning, their location noted, and the refugium was rotated 180° degrees. Rotation helped control for unknown environmental factors other than prior occupancy that may affect choice. The cups containing the seeond set of spiders were then uncapped and the cardboard refugia placed over open cups, onee again forcing the spider to make a choice inside the refugia. Twenty spiders were initially tested with 18 choosing an angle (16 always in the same location in a 60° angle each day, qualifying these for the second half of the assay) to which a second cohort of 16 spiders was subsequently exposed. The second cohort of spiders was checked for choice the next day.

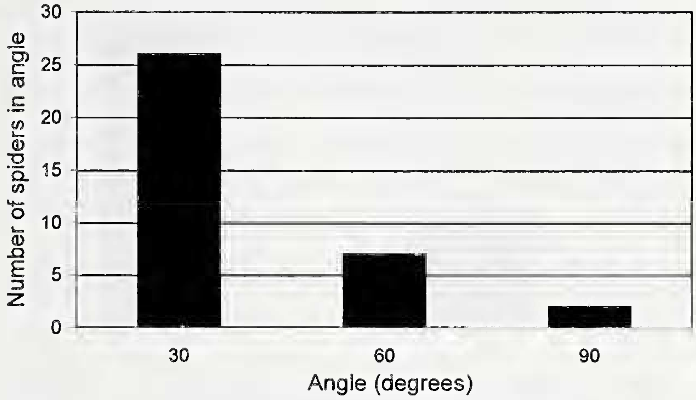

Figure 7.—Preference of brown widows for angles of 30°, 60° and 90°.

Statistics.—A Chi-square Goodness of Fit Test at the 0.05 level of significance was performed on all assays to determine refugia preferences. Data were analyzed using the statistical package SAS.

RESULTS

Angle preference assay.—In the angle preference assay, 35 of the 48 brown widows chose a refugium in the cardboard triangle with a significant preference for 30° angles ($\chi^2 = 27.49$, df = 2, $P < 0.001$; Fig. 7); the remaining 13 settled elsewhere in the insect cage. The angle with the staple was chosen 13 of 35 times (37.1%) indicating that presence of the staple had no influence on the choice of angle ($\chi^2 = 0.22$, df = 1, $P = 0.63$)).

Depth preference and site fidelity assay.—In the depth preference assay, 28 of 36 spiders chose one of the cavities in the cardboard box with the remaining eight choosing folds of the insect cage. Spiders choosing cavities preferred a depth of 75 and 100 mm depth over 25- and 50-mm depths ($\chi^2 = 17.43$, df = 3, $P = 0.001$) (Fig. 8).

In the site fidelity assessment, all 28 spiders that chose a cavity in the cardboard, remained in the same depth and even in the same corner within that cavity for each of the seven days that they were checked. Likewise, the eight spiders that chose a location other than inside the cardboard box occupied the same location every day that they were checked.

Surface roughness assay.—In the surface roughness assay, 48 spiders were tested of which 37 chose a cavity in the cardboard. The cavity lined with corrugations was preferred nearly 3:1

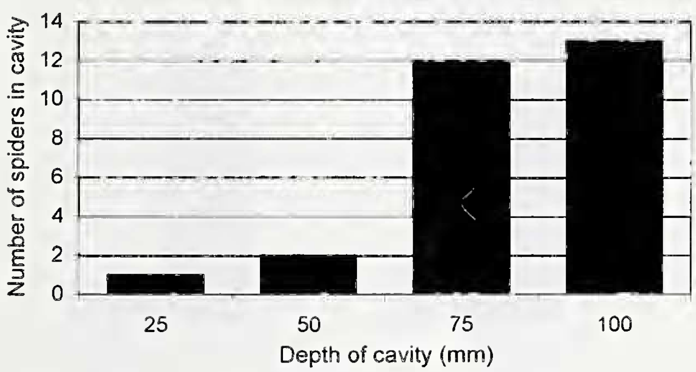

Figure 8.—Preference of brown widows for depths of 25, 50, 75 and 100 mm.

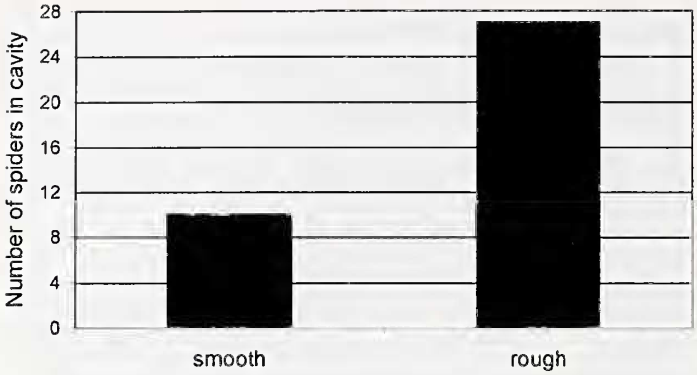

Figure 9.—Preference of brown widows for rough vs. smooth surfaced cardboard.

over the smooth-walled cavity (χ^2 = 7.81, df = 1, P = 0.005; Fig. 9).

Pre-occupancy assay #1.—Of the 32 spiders of the first cohort that were tested, only 15 chose the cardboard refugia with 13 choosing 60° angles and two choosing 120° angles (χ^2 = 8.07, df = 1, P = 0.005). Regarding the 13 pre-occupied 60° angles, the second cohort of spiders chose the pre-occupied angle 12 times and one spider chose the uninhabited 60° angle, a significant difference (χ^2 = 9.31, df = 1, P = 0.002) (Fig. 10). Of the remaining two choices where the 120° angles were chosen by the first set of spiders, the second cohort chose 60° angles instead of choosing the pre-occupied obtuse angle. Even though only 15 of 32 spiders (47%) chose cardboard refugia in the first round, all 15 spiders in the second round that were tested on previously-inhabited refugia chose one of the angles in the cardboard.

Pre-occupancy assay #2.—In the second assay of 20 spiders, 17 chose a 60° angle, one chose a 120° angle (χ^2 = 14.22, df = 1, $P < 0.001$), and two did not leave their cups. Of the 17 that chose a 60° angle, 16 were in the same location for each of the seven days that they were checked. One spider moved from one 60° angle to the other on day 2 and then moved back on day 7; this refugium was not tested in the second cohort release nor was the refugium where the 120° angle was chosen. The second cohort of 16 spiders chose the previously occupied 60° angle 14 times with one spider choosing the unoccupied 60° angle and another choosing a 120° angle (χ^2 = 9.0, df = 1, P = 0.003; Fig. 10).

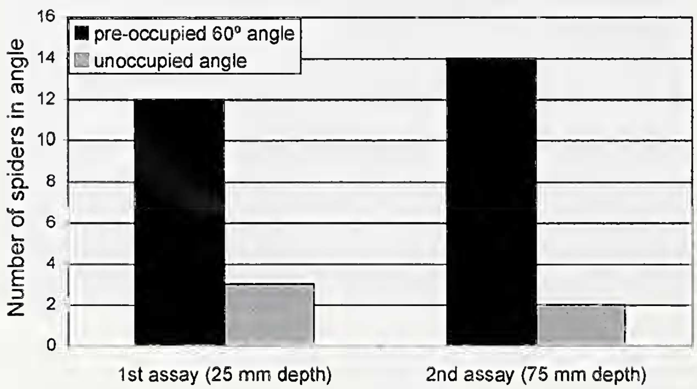

Figure 10.—Preference of brown widows for previously occupied refugia.

DISCUSSION

Brown widows demonstrated a distinct preference for refugia with acute angles, deep cavities, rough surfaces and previously occupied by a conspecific. Experimental spiders appeared to examine their environment and actively made decisions as to where to establish refugia. An extensive body of research exists regarding habitat selection in spiders, mostly performed in natural settings, examining the biotic and abiotic factors that spiders find attractive for web sites (e.g., reviews in Janetos 1986 and Gillespie 1986). Most of these studies involved localized ecological aspects such as complexity or height of vegetation, proximity to surface water, or availability of silk attachment sites (e.g., Gillespie 1987; Herberstein 1997). Some studies involved manipulation of the environment by removal or enhancement of vegetation (e.g., McNett & Rypstra 2000) whereas others introduced experimental structures into an environment to investigate how spiders would use physical enhancements (e.g., Robinson 1981; Stropa 2010). Fischer & Vasconcellos-Neto (2005) reported on *Loxosceles intermedia* Mello-Leitão, 1934 and *L. laeta* in synanthropic situations in Brazil and found the spiders had a preference for placing their retreats on rough surfaces such as paper, wood and construction materials rather than plastic or metal. Riechert (1976) reported on the importance of various natural environmental features for web site acceptance of the desert spider, *Agelenopsis aperta* (Gertsch, 1934).

In this current study, the brown widow's choice of the smallest acute angle in the triangle assay reflects what is observed in urban environments. However, in the absence of easily accessible alternatives, brown widows will exploit 90° angles on the undersides of picnic tables and other locations because a 90° angle is a common by-product of human-built structures. Brown widows typically make long, thin conical retreats that would fit snugly into a 30° angle. The choice of deeper cavities was somewhat unexpected because, in collections for earlier studies (Vetter et al. 2012a, b), great variation in web sites was noticed in the field, including some that were extremely exposed such as the underside of wrought iron railings where, when backlit by the sun, the entire silhouette of the spider could be detected (R. S. V., pers. obs.). Rough surfaces might be easier for fastening webbing and thus provide a stronger conical retreat. However, spiders easily attached silk to smooth surfaces in the other assays. The attractiveness of sites previously occupied by conspecifics in brown widows parallels similar behavior in other web-spinning spider species (Leborgne & Pasquet 1987; Hodge & Storfer-Isser 1997; Vetter & Rust 2008). Spider silks are proteins with metabolic costs associated with their production (Craig et al. 1999); some spiders, such as araneids, reingest their webs (Janetos 1986). Such a behavioral preference for pre-occupied retreats should enable conservation of silk proteins. This behavior also may explain the few occasions when Vetter et al. (2012a, b) discovered up to 35 brown widow egg sacs in one location. The extreme number of egg sacs is likely the work of more than one female, although brown widow females can oviposit more than 20 egg sacs in a lifetime (Bouillon & Lekic 1961; Heeres 1991).

Brown widows are synanthropic as they are often found around homes and urban areas as opposed to more natural environments (Baerg 1954; Lamoral 1968; Vetter et al. 2012b; Marie & Vetter 2015). Our findings might be useful information for the pest control industry. Knowing where brown widows may be more likely to choose habitats for making a retreat could focus pest management efforts. Our information on web site preferences may also reduce envenomation risk for the general public by increasing awareness of the spider's presence on patio furniture or other yard structures (e.g., fence railings, looped door handles).

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